An Underwater Bioluminescence Assessment Tool (U-BAT)

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LONG-TERM GOALS

Our long-term objective for Phase II of our research is the construction of four Underwater Bioluminescence Assessment Tool (U-BAT) commercial prototypes that have been fully characterized in the laboratory and field, thereby paving the way for widespread use and acceptance of the U-BAT to measure bioluminescence by oceanographers and the Navy. WET Labs will transition the technology, developed at UCSB, into a commercially available version that is small, light weight, platform-adaptable and couple it with already proven key bio-optical instruments to provide a single system for wide-scale, cost effective, full water column profile biological discrimination. The envisioned U-BAT will directly address Naval survey and tactical operations in providing a visibility and vulnerability assessment for deployed assets and potential threats. By broadening the use of bioluminescence (BL) measurements U-BAT will significantly increase general understanding of the roles of BL in oceanic biodynamics. This work directly addresses ONR topic # N05-T026 for the need to transition new and novel BL sensing technologies from the research to the commercial realm in order to enable a more comprehensive quantification of the spatial and temporal variability of biogeochemical complexity in coastal and oceanic ecosystems.

OBJECTIVES

The primary objective for the STTR Phase II project is to produce four commercial prototypes of the Underwater Bioluminescence Assessment Tool (U-BAT), a general purpose commercial bathyphotometer for biological assessment of natural waters evolved from the Multipurpose Bioluminescence Bathyphotometer (MBBP-G3) technology developed at UCSB. Our goals for this phase of the STTR Phase II project were: 1) to build 2 U-BAT prototypes from the assimilated mechanical, electrical and control code designs of the MBBP-G3 technology, 2) to evolve the MBBP-G3 design to simplify commercial manufacturing and to incorporate design improvements defined by a core group of bioluminescence researchers, 3) to test the performance of U-BAT in the laboratory and field, 4) to determine the utility of integrating other Inherent Optical Properties (IOPs) with the U-BAT sensor, 5) to provide calibration technology and methodology for measurement traceability.

APPROACH

A key asset for the design and development components of the final U-BAT product is the involvement of a core group of researchers in the field of bioluminescence. Drs. James Case (UCSB), Mark Moline (Cal Poly), Edith Widder (Ocean Research & Conservation Association), Steve Haddock (MBARI), Mike Latz (Scripps), and Mark Geiger (NAVO) compose our academic and Naval partners. Continued involvement of our partners in prototype testing will play a crucial role toward the

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14. ABSTRACT

Our long-term objective for Phase II of our research is the construction of four Underwater Bioluminescence Assessment Tool (U-BAT) commercial prototypes that have been fully characterized in the laboratory and field, thereby paving the way for widespread use and acceptance of the U-BAT to measure bioluminescence by oceanographers and the Navy. WET Labs will transition the technology, developed at UCSB, into a commercially available version that is small, light weight, platform-adaptable and couple it with already proven key bio-optical instruments to provide a single system for wide-scale, cost effective, full water column profile biological discrimination. The envisioned U-BAT will directly address Naval survey and tactical operations in providing a visibility and vulnerability assessment for deployed assets and potential threats.

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development of the commercial U-BAT. As the MBBP-G3 technology is evolved into the commercial product, frequent design review meetings are essential to communicate and incorporate design changes to ensure that the U-BAT will provide the naval and oceanographic community with a single system for wide-scale, cost effective, full water column profile biological discrimination. Extensive trials coupled with iterations of system modifications are critical to the overall success of the Phase II project in that they will help define the desired range and capacity for the commercial U-BAT product.

WORK COMPLETED

The first 2 U-BAT prototype-1s have been built and preliminary tests in both the laboratory and the field have been completed. As we review the results from the first round of laboratory and field tests, WET Labs engineers are working to modify the U-BAT design to incorporate "lessons learned" to reach our next goal: the production of 2 second generation U-BAT commercial prototypes (U-BAT prototype-2). During the Phase II award period the following was accomplished:

1. Motor design workshop held at UCSB (December, 2006)
John Koegler, WET Labs mechanical engineer, worked with Cyril Johnson at UCSB to evolve the MBBP-G3 motor technology. As a first step toward achieving a unified electronics BUS, an objective of this project, discussions on re-designing the motor occurred, in which motor control would be moved from the mid-body section to the PMT-housing. Since the motor and impeller design is so unique, photographic documentation on the MBBP-G3 pump and flow meter construction was also completed at this time.

2. Electronics design workshop held at UCSB (April 2007)
Wes Strubhar, WET Labs electronics engineer, worked with Cyril Johnson at UCSB to unify the PMT circuit, motor circuit and calibration light source onto a single electronics board set. Design advances were the incorporation of back-EMF sensing brushless DC motor controller into the electronics system and an upgrade to Microchip Technology PIC 18F4520 for increased processing power in communication capabilities.

3. Two U-BAT prototypes built and preliminary lab tests complete (July 2007)
WET Labs team (John Koegler, Wes Strubhar, and Cris Orrico) traveled to UCSB to complete the U-BAT builds under the direction of Cyril Johnson. During this time, pump and flow rates were characterized, the sensor was calibrated using the Optronics light source, PMT response linearity was determined, and biological samples were used to test the response of the U-BAT sensor to fine-scale bioluminescence signals.

4. U-BAT field deployment (August 2007)
A side-by-side comparison of U-BAT, MBBP-G3, MBBP-G2 was conducted at the Cal Poly test facility in Avila, CA. All three bathyphotometers were installed on the Cal Poly autonomous profiler along with chlorophyll fluorescence, turbidity (WET Labs ECO-FLNTU), scattering (WET Labs C-star) temperature and salinity (Sea-Bird Electronics CTD) sensors. From August 8-14, 2007, nighttime profiles were collected every ½ hour and discrete water samples approximately every hour to test for possible differences between MBBP-G3 and U-BAT capture efficiency.

RESULTS

Two U-BAT prototype-1 builds have been completed. The primary embodiment of the U-BAT prototype-1 sensor system was compiled from detailed knowledge of the MBBP-G3 design technology and the input solicited from a core group of active marine bioluminescent research scientists and naval partners. The development of this primary embodiment design enabled WET Labs to produce the preliminary specifications for the construction of the first 2 U-BAT prototypes, completed for our Phase II work. Extensive trials coupled with iterations of system modifications will help define the desired range and capacity for the second U-BAT prototypes and the final commercial U-BAT product. The U-BAT prototype-1 design has focused on integrating the electronics into a single, scalable BUS, reducing the complexity in the form factor of the external housing. These changes will move us toward achieving our Phase II goal: To produce a scalable (in terms of sensing parameters and capabilities) commercial bioluminescence sensor that can be integrated into a variety of coastal ocean platforms including AUV's, moorings, and vertical profilers for general oceanographic and naval use.

In order to reduce the complexity of the overall design, and to decrease the machining time, a modular design was developed. Modularity of the mechanical design will simplify access to component parts for instrument cleaning and calibration. Unification of the electronics components will reduce the number of cables needed to control instrument components, improve component control, and simplify access to electronics during factory servicing.

As a first step toward unifying the electronics system, an Allegro A8940 three-phase brushless DC motor driver/controller has been incorporated into the motor system. It uses back-EMF sensing to detect the rotor position to control the commutation timing, thus eliminating the need for Hall Effect sensors. This provides an advantage, as the motor controller may be moved into the PMT housing section, which will make servicing the motor controller and photomultiplier (PMT) control boards straightforward. A breadboard was designed to test the efficiency of the motor driver and to test various filter components needed to obtain stable speed control in water. Pump stability is the crucial source of stimulation that causes bioluminescent organisms to flash. Any variation in the pump could directly cause a variation in bioluminescence signal. The system design is optimized for stable speed control in water, which is the primary function for the motor controller. As the motor controller design evolves, the small size of the back EMF sensor is a great advantage, and even smaller control components are available in the same family.

Following the expectations for a unified electronics BUS and the possibility for system expansion, the U-BAT microcontroller was upgraded to a PIC 18F4520 microcontroller. This modification will improve the communication stream and simplify interconnections between system components. The new microcontroller is of the same family as the PIC chip used in the MBBP-G3, but has more pins which will allow the system to run faster and incorporate more memory for expanded functions. For example, in the MBBP-G3 communication was daisy chained from the pump to the PMT and was conducted across 6 connections. Incorporation of the new microcontroller and the modifications to the electronics design, discussed above, components will be able to communicate separately over RS485 and with each other over 3 bi-directional connections which will improve system speed.

A review of the MBBP-G3 components by WET Labs engineers and machinists revealed that although all of the mechanical components can be fabricated at WET Labs, some of the current parts are laborious and intricate to manufacture. However, vital components necessary to maintain the

consistency of measurement characteristics were not altered. Thus design changes to alter some of the non-crucial sensing components (such as the shape of the body) were necessary so that the WET Labs machine shop can more rapidly and efficiently manufacture these parts. One part in particular, the intake nozzle, would be laborious to machine due to the complex external curvature of the part. In reviewing the part design with Cyril Johnson, the critical component of this part is the S-shape of the interior of the intake nozzle which must satisfy opposing requirements for ambient light baffling and minimal premature mechanical excitation of bioluminescence. However, the external shape bears little effect on the overall performance of the sensing system and was modified to reduce machine time. Figure 1 shows the U-BAT prototype-1 components after completed machining, prior to assembly.

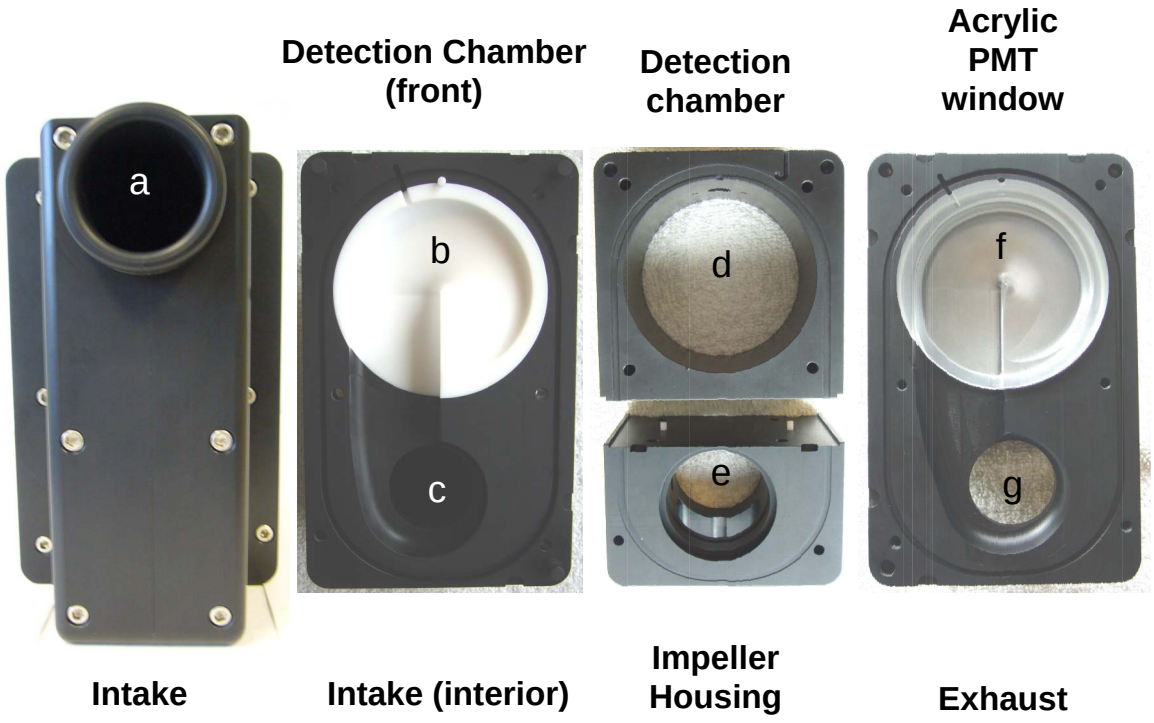

Figure 1: U-BAT prototype 1.

Upon completion of all the prototype components, a WET Labs team traveled to UCSB to assemble and test the first two U-BAT protype-1s under the direction of Cyril Johnson. During this time, preliminary tests of the sensor flow rates were conducted in the laboratory and the flow rate conversion factor was determined which is needed to convert flow RPM to mL/s. Both U-BATs were calibrated with the UCSB Optronics light source, the same method used to calibrate the MBBP-G3, to maintain consistency of measurement prior to the August field deployment. Analysis of PMT response linearity was conducted at 546 nm, the maximum light output of the Optronics source. At each gain (HV Step), the PMT response was measured at four different light intensities, controlled by aperture size (Table 1). Biological samples of *P.fusiformis* were injected at the intake of the U-BAT show that it can measure the same fine-scale bioluminescence structure as the MBBP-G3 (Figure 2).

Table 1: Linearity tests of U-BAT, showing strong exponential relationship across each high voltage steps.

HV step	Exponential relationship	r^2
HV Step 1	A/D counts = 0.086 $e^{2.49\ x}$	0.99
HV Step 2	A/D counts = 0.33 $e^{2.37\ x}$	0.99
HV Step 3	A/D counts = 1.22 $e^{2.31\ x}$	0.99

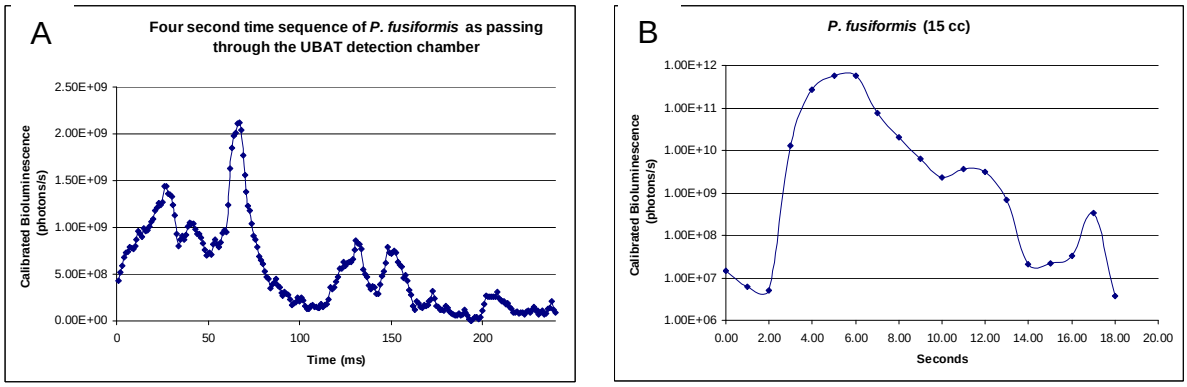

Figure 2: A. Four second time sequence of P.fusiformis as it passes through the U-BAT detection chamber, showing that U-BAT can capture the same fine-scale features as the MBBP-G3. Bioluminescence is reported in calibrated units (photons/s) and time (ms). B. Injection of 15cc P.fusiformis at intake shows that U-BAT has similar residence times to MBBP-G3.

From August 8-14, 2007 side-by-side comparison of U-BAT prototype-1, MBBP-G2, and MBBP-G3 was conducted at the Cal Poly pier, Avila, CA. The U-BAT was installed on the Cal Poly autonomous profiling system along with the following instruments: WET Labs fluorescence and turbidity sensor (ECO-FLNTU), WET labs C-star turbidity sensor, Sea Tech scattering sensor (LSS), Sea-Bird conductivity, temperature and depth (CTD) and plankton capture nets (Figure 3). Multi-parameter profiles were collected every ½ hour and discrete water samples were collected and preserved approximately every hour to test for possible differences between MBBP-G3 and U-BAT capture efficiency. Bathyphotometers were well correlated and measured the same bioluminescence structure. The utility of including additional measurement parameters on U-BAT is currently undergoing analysis and discussion. Since August, side-by-side bioluminescence profiles of MBBP-G2, MBBP-G3 and U-BAT have continued every ½ hour.

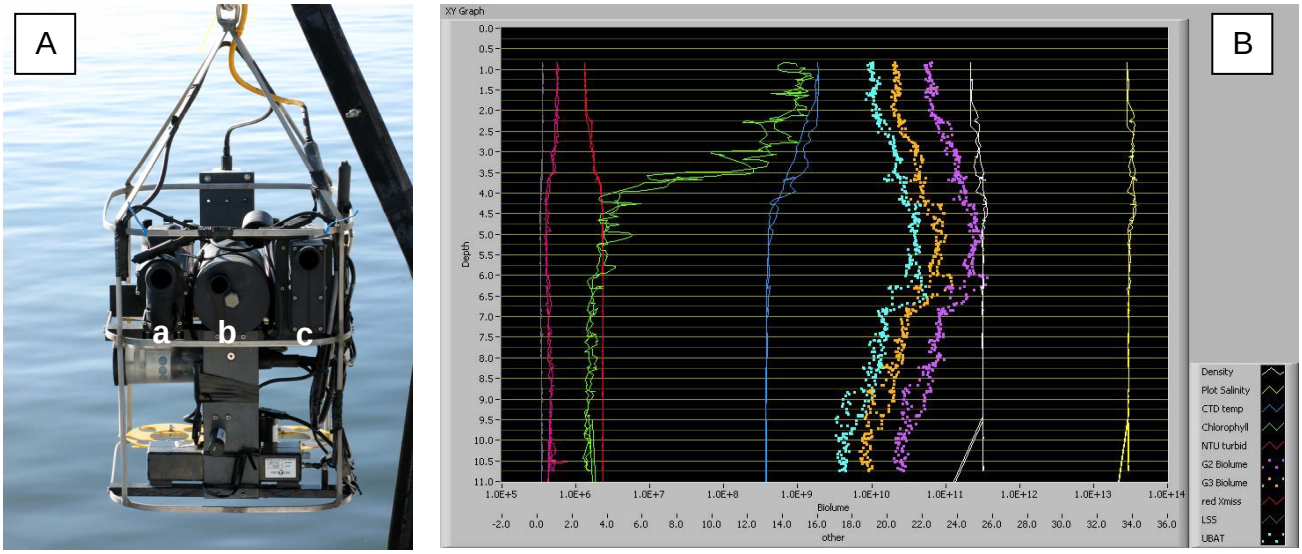

Figure 3: A. Cal-Poly autonomous profiler, with bioluminescence profiling pacakge attached. (a) MBBP-G3,(b) MBBP-G2 and (c) U-BAT are located side-by-side. Plankton capture devices are installed on the MBBP-G3 and the U-BAT to allow for plankton capture efficiency analysis or spur of the moment plankton collection (nets not attached in this picture). B.Verticle profile (depth in meters) collected August 10, 2007 at 20:23:19, where bioluminescence from the U-BAT (cyan) MBBP-3 (gold), MBBP-G2 (puple) is well correlated.

Following, both laboratory and field tests Cyril Johnson reviewed the design and performance of U-BAT prototype-1. WET Labs engineers were able incorporate significant improvements into the U-BAT prototype-2 design. Figure 4 shows the evolution in mechanical design from the current version to the prototype-2 revision. Of importance was the elimination of the external connector from the mid-section of the U-BAT prototype-1 body (Figure 4, Panel 2, part a, compared with Panel 4 part a). A MCBH-type connector was used to connect the motor module with its control board, located in the PMT housing. This mechanical design change was made possible by the unification of electronics now all housed in the PMT section. The U-BAT body was re-designed to reduce the amount of material, thus reducing sensor weight by 6 pounds. To improve access to the U-BAT and mounting, the number of bolts was reduced and mounting points added. A copper ring will reduce the bio-fouling that commonly occurs at the sensor exhaust (Figure 4, Panel 2 part d compared with Panel 4 part d). It is essential to stress that the design of components vital to stimulation and measurement of bioluminescence have not changed from the MBBP-G3 design. These include the helical ramp entrance and exit to the detection chamber, the size and shape of the detection chamber, the size and shape of the pump and flow impellers, and the PMT. WET Labs machine shop engineers have been involved in the mechanical revisions and expect that production of the new U-BAT prototype-2 design will require less machine time and all components can be made onsite at WET Labs.

Mechanical design changes have also been made to improve internal components. The pump-flow motor is now a modular component, separate from the U-BAT mid-section that fits within a uni-directional keyed slot (Figure 4, Panel 2, part b compared to Panel 4, part b). Since the motor control and processing have moved to the PMT housing, this part is considered expendable, if it should fail. In the U-BAT prototype-1 design if a motor failure were to occur the entire mid-section would need replacement because the motor is potted into the mid-section. The light baffle exhaust has also been re-

designed to reduce manufacturing time (Figure 4, Panel 2, part c compared to Panel 4, part c). Rather than milling light baffle waves, which are time intensive, a helix-inspired light baffle system is used. Each section of the baffle can be milled easily and the set of light baffles can be replaced easily if needed. John Koegler and Cyril Johnson are currently discussing materials to use for the baffles, such as copper painted black to reduce light scattering.

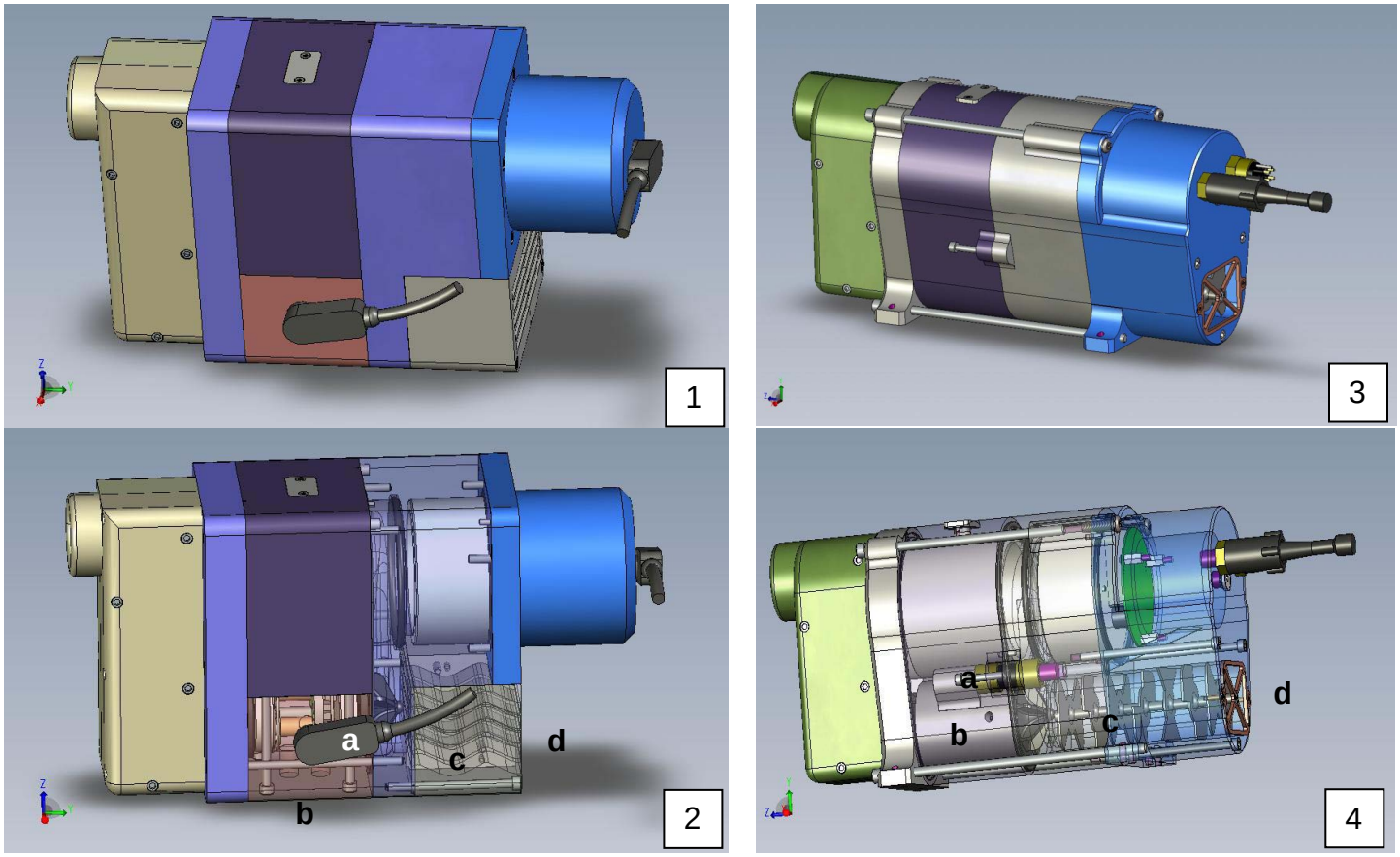

Figure 4: Comparison of U-BAT prototype version evolution. Panel 1 shows the external body of U-BAT prototype-1 with external motor cable at mid-section. Panel 2 shows the external body of the U-BAT prototype-1 (a) external motor connector, (b) potted motor, (c) exaust wave-baffle and (d) exaust port. Panel 3 shows U-BAT prototype-2 external embodiment design. Panel 4 shows trasnparent sections of U-BAT prototype-2 evolved design (a) the modular, expendable motor with (b) internal connector, (c) improved light baffle design, and (d) copper ring to reduce bio-fouling at exaust.

IMPACT/APPLICATIONS

While bioluminescence has long held promise as an important tool for Naval operations and biological research and monitoring, thus far there have been no commercially available tools for wide scale measurement and dissemination of results with a standard methodology. The bioluminescence research community is currently in a situation in which individual research groups tend to adopt or develop unique measurement solutions depending upon applications, available resources, and other factors. This in turn has created a scenario in which there is virtually no common denominator

measurement against which individual researchers can compare results. Our goal in developing the U-BAT sensor is to ensure a common bioluminescence measurement baseline that is well characterized in terms of sensor stability, accuracy, and response and that is inter-calibrated with Naval bioluminescent standards.

We believe that the U-BAT technology will fulfill not only a DoD need, but also a need within the academic marine research realm by providing these communities with a light-weight, inexpensive bioluminescence sensor capable of accurately measuring fine-scale vertical bioluminescence potential (<< 1 m resolution). In addition, the calibration methodology defined in Option I, the U-BAT will be well characterized and intercomparable with data collected using the MBBP-G3. With special attention to the needs of the operational Navy, the U-BAT will provide a modern, commercially available instrument to collect bioluminescence data to incorporate into the Navy database, needed to assess the possible risk of flow-stimulated bioluminescence. During the bioluminescence technology review meeting, held December 12, 2005 at UCSB, many potential applications and markets for the commercial sector were discussed by a core group of bioluminescence researchers, which include Mark Moline, Steve Haddock, Jim Case, Edith Widder, and Mike Latz. Several potential applications presented themselves and thus provide a framework on how best to approach and test the final U-BAT design for commercial production. As the U-BAT is used in the field to collect bioluminescence data, our academic partners will begin to develop data end products using the combination of optical and bioluminescence measurements to 1) better assess biogeochemical variables, 2) integrate measurements for a more robust prediction of bioluminescence water-leaving radiance, 3) refine the use of bioluminescence as a measurement that can distinguish trophic groups, and 4) relate bioluminescent signals to taxonomically identified sampled organisms. It is anticipated that the data end products developed by our academic partners will aide and make the case for the transitioning the U-BAT package for routine deployment by the oceanographic community. We expect commercial availability of this instrument will greatly accelerate the general undestanding of marine bioluminescence.

TRANSITIONS

The first 2 prototypes have been constructed and tested. We are currently analyzing data from the first round of sensor tests, conducted with our primary academic partners UCSB and Cal Poly. A final design review of U-BAT prototype-2 will occur on October 9, 2007 at WET Labs. Upon acceptance by the engineering team, 2-D drawings will be created and part fabrication will begin within two weeks. A second round of protoype tests, with U-BAT prototype-2 will involve both primary and secondary academic partners.

RELATED PROJECTS

Since testing and defining operational capability are an ongoing aspect of the project, numerous deployments coupled with iterations of system modifications are the key to specifying the final operational capabilities of U-BAT. We view this next period of extensive trials as critical to overall success of the Phase II project. Ideally this testing would also be coordinated with ongoing Naval efforts.